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The C3(1)/SV40 T-antigen transgenic mouse model of mammary cancer: ductal epithelial cell targeting with multistage progression to carcinoma

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The 5′ flanking region of the C3(1) component of the rat prostate steroid binding protein (PSBP) has been used to successfully target the expression of the SV40 large T-antigen (Tag) to the epithelium of both the mammary and prostate glands resulting in models of mammary and prostate cancers which histologically resemble the human diseases. Atypia of the mammary ductal epithelium develops at about 8 weeks of age, progressing to mammary intraepithelial neoplasia (resembling human ductal carcinoma *in situ* [DCIS]) at about 12 weeks of age with the development of invasive carcinomas at about 16 weeks of age in 100% of female mice. The carcinomas share features to what has been classified in human breast cancer as infiltrating ductal carcinomas. All FVB/N female mice carrying the transgene develop mammary cancer with about a 15% incidence of lung metastases. Approximately 10% of older male mice develop anaplastic mammary carcinomas. Unlike many other transgenic models in which hormones and pregnancy are used to induce a mammary phenotype, C3(1)/Tag mice develop mammary tumors in the mammary epithelium of virgin animals without hormone supplementation or pregnancy. Although mammary tumor development appears hormone-responsive at early stages, invasive carcinomas are hormone-independent, which corresponds to the loss of estrogen receptor-α expression during tumor progression. Molecular and biologic factors related to mammary tumor progression can be studied in this model since lesions evolve over a predictable time course. Genomic alterations have been identified during tumor progression, including an amplification of the distal portion of chromosome 6 containing *ki-ras* and loss of heterozygosity (LOH) in other chromosomal regions. We have demonstrated that stage specific alterations in the expression of genes which are critical regulators of the cell cycle and apoptosis are functionally important *in vivo*. C3(1)/Tag mice appear useful for testing particular therapies since growth of the mammary tumors can be reduced using chemopreventive agents, cytokines, and an anti-angiogenesis agent. *Oncogene* (2000) **19,** 1020–1027.

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Introduction

The application of transgenic technology to study mammary gland biology and cancer has proven extremely powerful for understanding important principles of transformation and for modeling cancer in a rodent system (Furth *et al*., 1998; Macleod and Jacks, 1999; Merlino, 1994). Numerous transgenic studies, including those reviewed in this journal issue, have demonstrated how perturbations in the expression of oncogenes, growth factors, and receptors may alter normal mammary development and lead to cancer.

The vast majority of these models have been developed using either the mouse mammary tumor virus (MMTV) or whey acidic protein (WAP) promoter/enhancer to direct heterologous gene expression to the mammary gland (see Cardiff *et al*., 2000, this issue; Amundadottir *et al*., 1996). Transcriptional activity of the MMTV and WAP promoters is often dependent on and highly responsive to pregnancy or administration of hormones (Pittius *et al*., 1988; Cato *et al*., 1989). Pregnancy dependent transgenic models of mammary cancer are dissimilar to human breast cancer in which pregnancy has been identified as a protective factor. This suggests that transcriptional activity of MMTV or WAP tends to be higher in hormone-stimulated, differentiated mammary epithelial cells. For this reason, these promoters are not well suited to study the influence of hormones on mammary oncogenesis since hormones will modulate the levels of transgene expression. In addition, expression driven from the MMTV promoter is often sporadic throughout the mammary gland (Stamp *et al*., 1992). Therefore, the development of additional hormone-independent promoters to direct expression to the mammary epithelium will be advantageous for transgenic modeling in the mammary gland.

Characterization of the mammary phenotype in transgenic mice carrying the PSBP C3(1) 5′ flanking sequence to drive expression of Tag has revealed interesting features relevant to the study of human breast cancer (Maroulakou *et al*., 1994; Shibata *et al*., 1996a, 1999). Since the C3(1) vector appears to target expression to the mammary epithelium differently than the MMTV or WAP promoters, the resulting mammary tumor phenotype has some unique features.

Originally, the C3(1)/Tag construct was designed to direct expression of Tag to the prostate epithelium of transgenic mice. C3(1)/Tag transgenic male mice, in

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fact, do develop phenotypic changes which highly resemble human prostatic intraepithelial neoplasia progressing to invasive carcinoma (Maroulakou *et al*., 1994, Shibata *et al*., 1996b). In addition, tumors of the urethral/bulbourethral and salivary glands were also observed in further detailed studies (Shibata *et al*., 1998a). However, 100% of female mice carrying the same transgene develop mammary carcinomas with some histologic similarities to human breast cancer. This model has been extremely useful for studying various aspects of mammary tumor progression, including changes in genomic organization, cell cycle regulation, apoptosis, and the influences of hormones on the natural history of tumor development. Chemoprevention agents, cytokine therapy, and an anti-angiogenesis agent have also been successfully used to suppress mammary tumor progression in the C3(1)/Tag transgenic mice.

Regulation of the rat prostate steroid binding protein C3(1) gene

In order to understand the transgenic expression pattern of the C3(1)/Tag transgene, it is helpful to review what is known about the regulation of the C3(1) gene, a subunit of PSBP. PSBP is the most highly secreted protein of the rat ventral prostate (Heyns and DeMoor, 1977). Previous transgenic experiments by Allison *et al*. (1989) demonstrated that all of the elements necessary to recapitulate the pattern of endogenous C3(1) expression in the rat are contained within an 8 kb genomic fragment which includes 4 kb of 5′ flanking sequence, three exons and 2 kb of sequence downstream of the polyadenylation site. Several *in vitro* studies have defined the location of various *cis*-acting elements, including androgen response elements (AREs). Although it has been shown that androgen receptor binds to elements contained within the promoter region as well as in the first intron (Rushmere *et al*., 1987), only the ARE contained within the first intron has been shown to be functionally responsive to androgen resulting in increased levels of transcription (Parker *et al*., 1987; Claessens *et al*., 1990; Tan *et al*., 1992). Studies in which up to 8 kb of the C3(1) 5′ flanking sequence were fused to the Cat reporter gene did not demonstrate any transcriptional response to androgen in co-transfection experiments (Parker *et al*., 1987; Claessens *et al*., 1990; Tan *et al*., 1992). There is no evidence that estrogen is able to activate C3(1) transcription, although few studies have addressed this issue (Vanaken *et al*., 1996). The C3(1)/Tag transgenic construct which we developed contains 4.5 kb of the C3(1) 5′ flanking region without intronic sequences. Thus, it is possible that the expression of this transgene is regulated primarily by tissue-specific *cis*-acting elements contained within the 5′ flanking region rather than by responsiveness to androgens.

It remains unclear why this transgene expresses in the mammary epithelium when the rat PSBP gene was thought to be primarily prostate specific. It is possible that low levels of C3(1) expression may normally occur in the rat mammary gland.

Recent studies have suggested that the C3(1) gene is part of a family of proteins which shares common protein motifs with other genes including mammoglobin, uteroglobin, CC10, and male mouse salivary gland protein (Watson and Fleming, 1996). As a member of the mammoglobin family, it is possible that the C3(1) 5′ flanking region contains some *cis*-acting elements which target expression to the mammary gland. It is also possible that the transgene lacks regulatory elements contained downstream of the C3(1) transcriptional start site that act as tissue specific silencers that further define tissue specificity.

Other groups have also used various forms of C3(1) as a transgenic targeting vectors to overexpress polyoma middle T-antigen (Py-MT) (Tehranian *et al*., 1996) and Bcl-2 (Zhang *et al*., 1997). The C3(1)/Py-MT construct used a 4.4 kb fragment of C3(1) which included about 3 kb of the 5′ flanking sequence and the first intron. Py-MT was expressed in the mammary and prostate glands as well as the lung, parotid glands and heart. The C3(1) regulatory regions used in the C3(1)/Bcl-2 construct were similar to those used in the C3(1)/Py-MT construct except it contained about 1.3 kb more 5′ flanking sequence. Bcl-2 expression was observed in the prostate, testes and uterus but was not examined in the mammary gland.

As discussed below, the C3(1)/Tag transgene is expressed primarily in the distal mammary ductal epithelium and terminal ductal lobular unit (TDLU) units leading to the progressive development of mammary cancers.

Action of SV40 Tag

The early region of SV40 produces both large T- and small t-antigen proteins involved in cellular transformation (Cole, 1998; Moens *et al*., 1997). Large Tag has been well characterized and used as a powerful transforming agent in numerous cell types both *in vitro* and *in vivo*. Although the expression of SV40 Tag has not been correlated to human breast cancer, studies have demonstrated that Tag-related transcripts may be expressed in mesotheliomas (Carbone *et al*., 1999) and human brains tumors (Martini *et al*., 1998), suggesting that Tag may be involved in the etiology of these tumors.

The mechanisms by which Tag induces transformation and tumorigenesis is thought to involve its ability to bind to and functionally inactivate p53 (Mietz *et al*. 1992) and Rb (Dyson *et al*., 1989), two tumor suppressor genes critical for maintaining homeostasis of cell growth. Many human cancers, including breast cancer, have been found to contain mutations or deletions which inactivate these tumor suppressor genes (Cox *et al*., 1994) leading to abnormalities in cell cycle regulation, increased cellular proliferation, and defects in the activation of apoptosis pathways. Thus, the Tag-induced transformation process involves the inactivation of critical growth control pathways that are also commonly altered in human cancers. This suggests that the induction of tumorigenesis by Tag in this transgenic model may utilize mechanistic pathways that are also involved in the development of human breast cancers.

Natural history and histologic progression of mammary tumors

The phenotypic abnormalities observed in the C3(1)/Tag mice correlate directly to the expression of Tag

(Figure 1; Maroulakou *et al*., 1994; Shibata *et al*., 1996a,b). Mammary tumor development is the predominant phenotype in C3(1)/Tag female mice, although female transgenic mice surviving beyond 5 months of age also occasionally develop mixed tumors originating from sweat glands located in the foot pads (Maroulakou *et al*., 1999), mild proliferative changes in the salivary glands (Shibata *et al*., 1998a), and occasionally tumors arising from the vomeronasal organ (Maroulakou *et al*., 1994).

The earliest observable mammary lesion is atypia of the ductal epithelial cells observed beginning at about 8 weeks of age (Figure 2a), suggesting the effect of Tag on nuclear morphology occurs quite early. These atypical cells likely represent the earliest stage of mammary intraepithelial neoplasia (MIN), low grade. As well as occurring in ducts, atypical epithelium can also be present in the TDLU (Figures 2d,e and 3a). Atypical lesions progress to high grade MIN beginning at about 12 weeks of age (Figure 2b,c). In prior publications we designated these lesions as nodular atypical hyperplasias (NAH). The hyperchromatic atypical cells lose polarity and form masses which extend into the duct lumen. Larger intraepithelial neoplastic lesions fill the duct lumen but do not invade the basement membrane; these lesions appear similar to human DCIS (Figure 2c). As seen in whole mounts, MIN may be focal or multifocal (Figure 3). MIN often progress into invasive carcinoma (Figures 2f–h and 3d) beginning at about 16 weeks of age at which time, tumors are grossly palpable. Carcinoma formation is multifocal with females over 5 months of age commonly developing several grossly palpable tumors. Tumors tend to arise earlier in the axillary glands, but all mammary glands are usually affected. Due to the rapid growth of large mammary tumors, females are euthanized by 7 months of age. Male and female mice heterozygous or homozygous for the transgene are fertile, but offspring from homozygous females must be fostered since mothers homozygous for the transgene are unable to lactate sufficiently to support their pups.

The lung is the predominant site for metastases in this transgenic model (Figure 2i), although metastases to the liver, adrenal and heart have been observed. Metastases to the brain, bone, and regional lymph nodes have not been observed demonstrating a major biologic difference between the behavior of the C3(1)/Tag transgenic mouse tumors and human breast cancer. As in the case of several other transgenic models, metastases to the lung are often of a vascular embolic type where metastatic lesions arise from hematogenously spread tumor cell emboli that generally grow within the blood vessels of the lung although they also invade vessel walls and lung parenchyma (Figure 2i). C3(1)/Tag mammary tumors have been observed to directly invade adjacent lymph nodes (Figure 2g), skeletal muscle (Figure 2h), salivary glands and skin.

Genetic variation between mouse strains can alter the natural history of tumor formation and metastases in mice carrying the C3(1)/Tag transgene. Tumor progression is 1–2 months slower in the C57B6 strain of mice compared to FVB/N mice carrying the C3(1)/Tag transgene. Whereas we observe a 10–15% incidence of lung metastases in the FVB/N background, over 50% of mice with the hybrid SV129 FVB/N background carrying the C3(1)/Tag transgene develop lung metastases (Maroulakou *et al*., 1997). These observations suggest that genetic modifiers are important modulators of phenotypic expression of the mammary tumors in this transgenic model.

Genomic changes during tumor progression

Numerous genomic alterations have been characterized in many human mammary tumors and have led to this discovery of genes involved in oncogenesis. These alterations may be observed as the gross loss or gain of chromosomal material, chromosomal rearrangements, localized deletions identified by LOH, or specific gene mutations.

Comparative genomic hybridization has revealed that mammary tumor progression in the C3(1)/Tag transgenic mice is associated with a gain of genomic material from the distal region of mouse chromosome 6 (Liu *et al*., 1998). We have demonstrated by Southern blot analyses that the *ki-ras* gene, which is

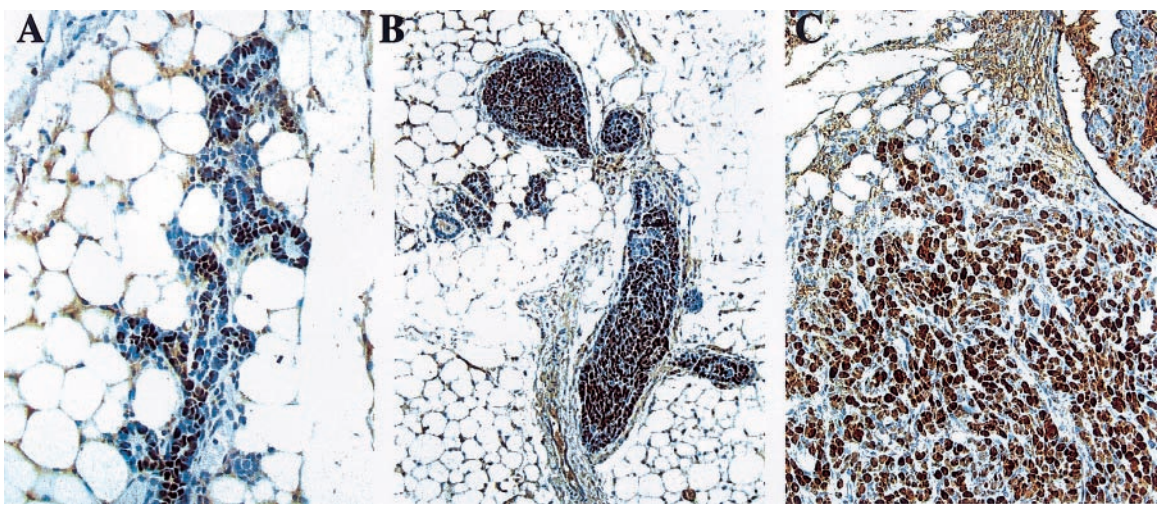

Figure 1 Immunohistochemistry of SV40 Tag expression during mammary tumor progression. (**a**) Expression in TDLU, (**b**) MIN (DCIS), and (**c**) invasive carcinoma

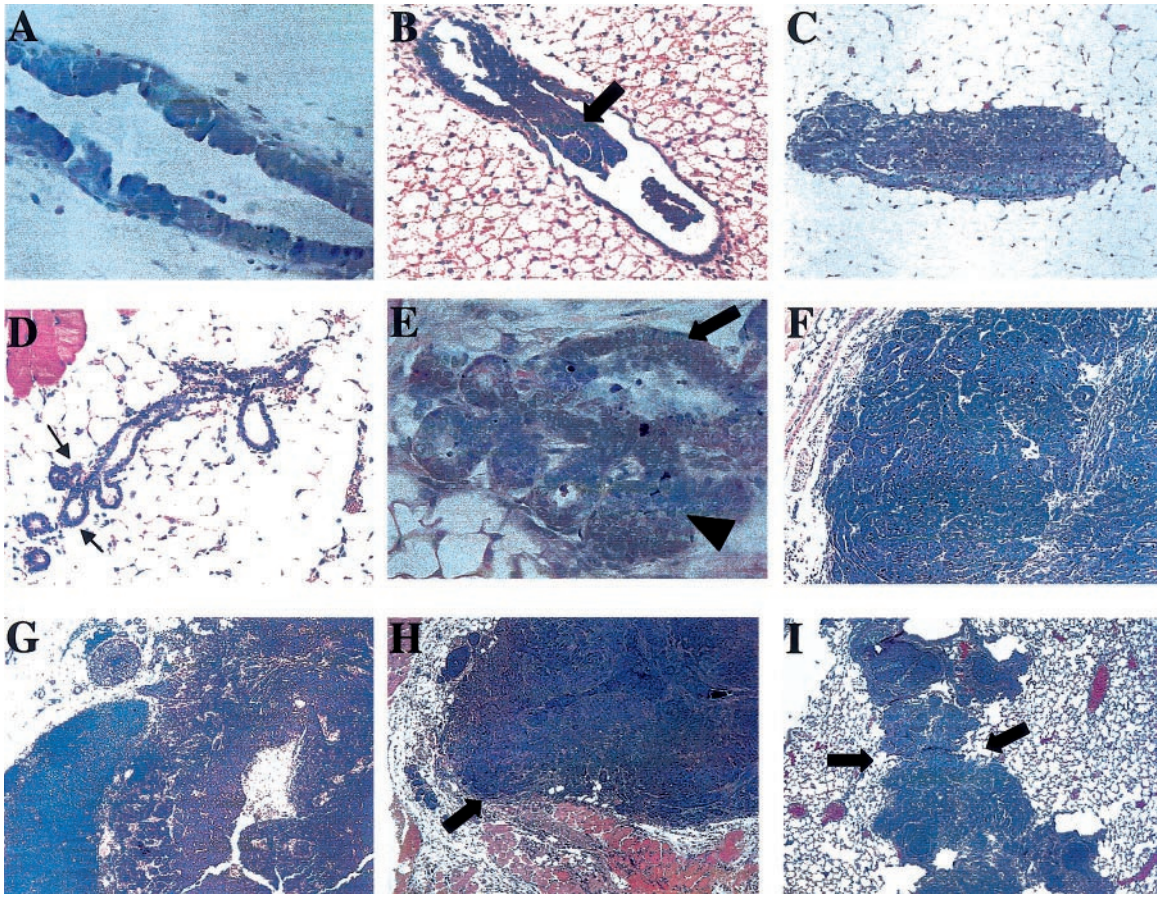

Figure 2 Progressive mammary lesions in C3(1)/Tag transgenic mice. (**a**) Mammary duct with region of cells demonstrating atypical ductal epithelium in an 8-week-old animal. Atypical cells are in a single layer; they are columnar, closely apposed and have large hyperchromatic nuclei. (**b**) Early mammary intraepithelial neoplasia (MIN) with a mass of proliferating atypical cells (arrow) extending into the ductal lumen. (**c**) More advanced MIN lesion with a mass of proliferating cells almost completely obliterating the lumen, but not invading through the basement layer of the duct. This lesion appears similar to human DCIS. (**d**) Minimal atypia in regions of the TDLU (arrows). (**e**) Higher magnification of a more severely affected TDU with atypia (arrow) and focal MIN (arrowhead) in a 12-week-old animal. (**f**) Invasive carcinoma with cribriform pattern in an 18-week-old animal. (**g**) Carcinoma directly invading into lymph node (LN) in a 20-week-old animal. (**h**) Carcinoma invading into muscle (arrow). (**i**) Embolic and invasive lung metastasis (arrows). (**a**–**i**) Hematoxilin and eosin. **a** and **e**, ×400; **b** and **c**, ×200; **d** and **f**) ×100; **g**–**i**) ×5

contained within this region of chromosome 6, is amplified up to 30-fold. Furthermore, the amplification of *ki-ras* correlates with increased expression of *ki-ras* RNA and protein and is associated with increased MAP kinase activity (Liu *et al*., 1998). In order to determine whether the amplification and overexpression of *ki-ras* is functionally important for tumor progression in this model, mice carrying the C3(1)/Tag transgene and lacking one allele of *ki-ras* were generated and studied. Through these studies we have demonstrated that *ki-ras* is indeed functionally important in accelerating mammary tumor progression. Mice lacking one normal allele of *ki-ras* have retarded tumor development compared to C3(1)/Tag mice with both wild type alleles of *ki-ras* which is not due to differences in strain background (Liu *et al*., manuscript in preparation).

Ongoing LOH studies indicate that several genomic loci on different chromosomes may harbor suppressor genes whose function is lost during mammary tumor progression in this transgenic model. Further studies using spectral karyotyping may reveal additional changes in chromosomal structure during tumor progression.

Molecular alterations during tumor progression

Tag is expressed in all tissues which develop proliferative lesions in the transgenic mice. RNA *in situ* hybridization and immunohistochemistry (Figure 1) demonstrate that Tag expression in the mammary gland is localized to the ductal epithelium and TLDU (Maroulakou *et al*., 1994; Shibata *et al*., 1996a). The levels of Tag protein appear to correlate directly with the levels of Tag mRNA. The entire early region of SV40 was used in the transgenic construct and contains both large T-antigen and small t-antigen (t). The appropriate RNA species for small-t antigen is made in the mammary epithelium. However, the role of small t in mammary tumor development in this model remains unclear, although it may be important for inducing tumor progression (Choi *et al*., 1988).

The mechanism for Tag-induced transformation includes the binding of this oncoprotein to two critical tumor suppressor genes, p53 and Rb, leading to their functional inactivation. Although p53 and Rb are functionally impaired by Tag, immunohistochemical studies of the C3(1)/Tag mammary tumors demonstrate that the absolute protein levels of p53 and Rb

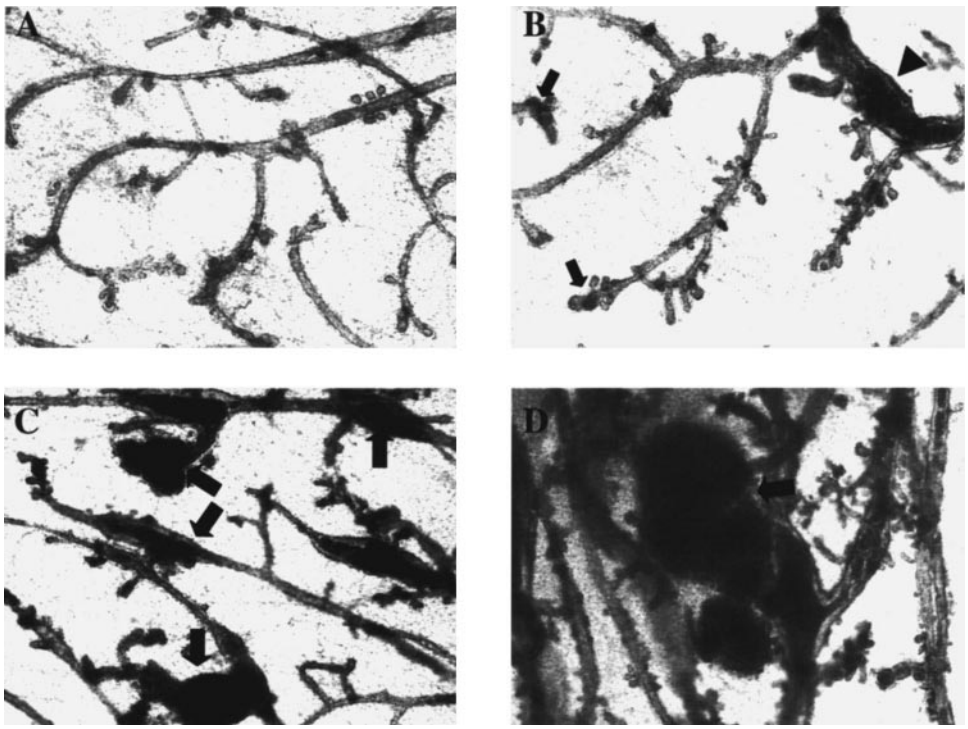

Figure 3 Whole mount analysis of progressive mammary lesion development. (**a**) Early proliferative lesions in TDU regions in an 8-week-old animal. (**b**) Increased number of TDU proliferative lesions (arrows) with larger ductal intraepithelial lesion (arrowhead) in a 14-week-old animal. (**c**) Increased number of ductal intraepithelial lesions (arrows) in a 17-week-old animal. (**d**) Large lesion (arrow), possibly invasive carcinoma in a 17-week-old animal. Carmine staining, ×5

increase within the nucleus in association with Tag. Functional inactivation of p53 and Rb impairs normal regulation of the cell cycle leading to increased proliferation and a loss of normal apoptotic responses. Tumor growth results from an imbalance between signals that stimulate cellular proliferation and those that result in the loss of cells through apoptosis. We have investigated additional changes in genetic pathways that regulate these two processes during mammary tumor progression.

Alterations in cell cycle regulation The inactivation of p53 by Tag leads to a loss of the cell cycle inhibitory functions of p21. This results in elevated levels of cyclins and cdk's (Figure 4). The increased expression of these key regulators of the cell cycle leads to increased cellular proliferation associated with tumor progression. Normally, in response to various signals, p53 is upregulated which stimulates an elevation in p21 expression ultimately resulting in the suppression of cyclins, cdk's, and cell cycle progression. However, in this Tag transgenic model, induction of p21 by p53 does not occur due to the binding of p53 to Tag. The elevated levels of cyclins and cdk's in the transgenic mammary tumors are very similar to those reported for human breast cancer (Figure 4, arrows). Recent gene therapy studies from our laboratory using an inducible retroviral delivery system have demonstrated that if p21 expression is restored in mammary tumor cells from the C3(1)/Tag transgenic mice, levels of cyclins and cdk's are reduced. When such cell lines are injected into nude mice and tumor growth is retarded when p21 expression is induced (Shibata *et al*., in preparation). This transgenic model may be useful for testing therapies targeted against key regulators of the cell cycle.

Bi-phasic apoptotic response during mammary tumor progression: a role for bax Under normal conditions, a protective apoptotic response may prevent damaged cells from accumulating and forming tumors. We examined how levels of apoptosis change during different stages of mammary tumor progression (Shibata *et al*., 1996a, 1999). Approximately 1% of normal adult virgin female mammary ductal epithelial cells are undergoing apoptosis when assayed using the TUNEL method. The rate of apoptosis, however, rises dramatically in the stages of atypia and MIN to about 13% of all cells within the lesions. The levels of apoptosis fall to about 5% as the early lesions transition into invasive carcinomas. Thus, at early stages of tumor progression, a protective apoptotic response occurs which is repressed as these lesions become more aggressive.

Since Bcl-2 family members have been shown to be key regulators of apoptosis, we have examined expression patterns for Bcl-2 family members during mammary tumor progression in this transgenic model (Shibata *et al*., 1999). The dramatic rise in apoptosis in the atypical and MIN lesions is associated with the elevated expression of the apoptosis inducer, *bax*. In order to test whether the expression of *bax* is, indeed, functionally significant in regulating tumor progression, we generated mice carrying the C3(1)/Tag transgene in a background lacking one or both alleles of *bax*. In C3(1)/Tag mice lacking one or both alleles of *bax*, tumor formation occurred earlier with increased tumor multiplicity, increased rate of

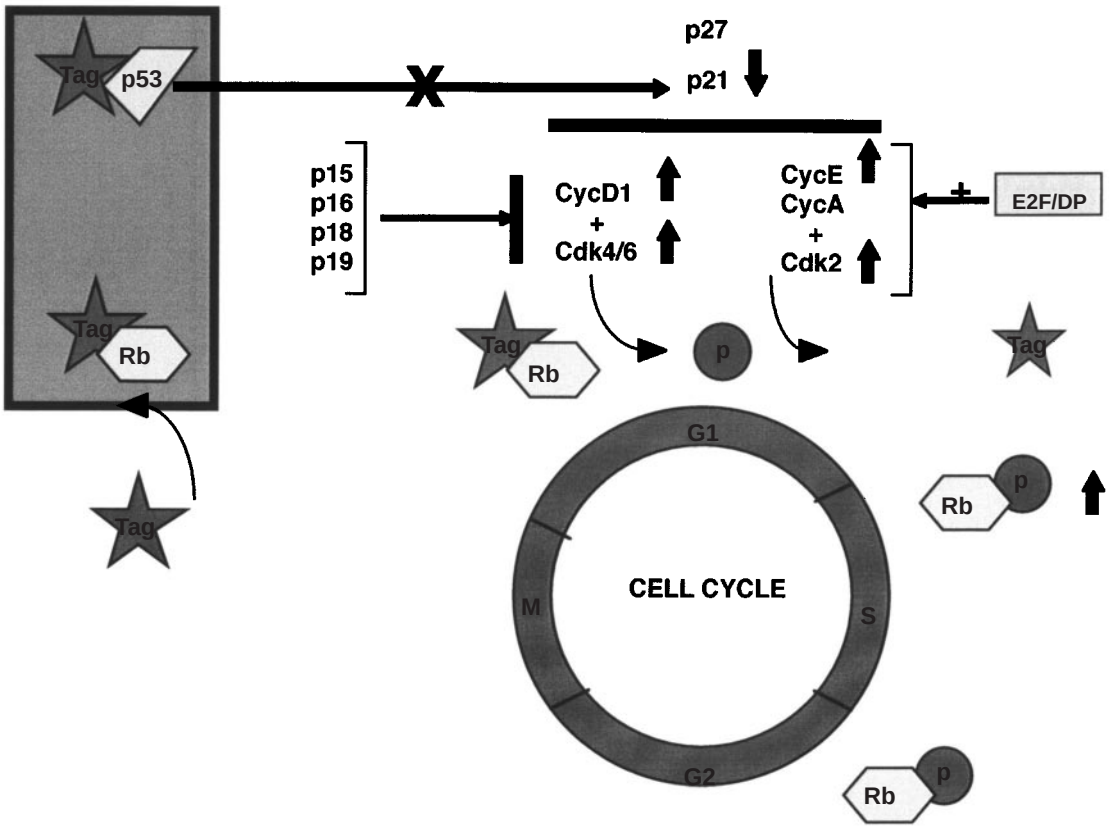

Figure 4 Cartoon summary of Tag-induced changes in expression of cell cycle regulators during mammary tumor progression. Functional inactivation of p53 reduces p21 expression which leads to elevation of cyclins/cdk's, and Rb phosphorylation (arrows)

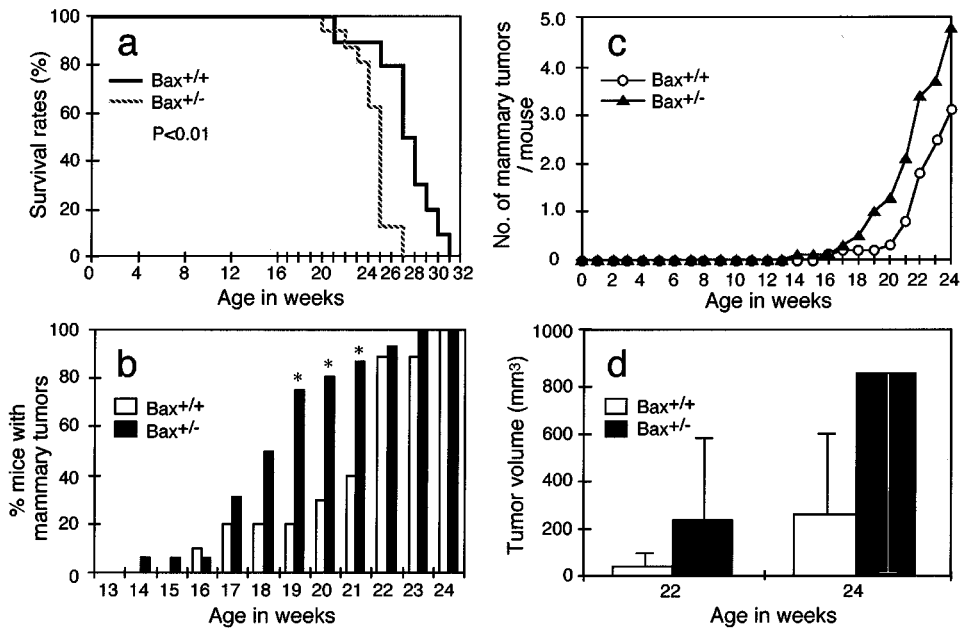

Figure 5 *bax* gene dosage effect on survival (**a**), incidence of palpable lesions (**b**), multiplicity of palpable lesions (**c**), and volume of the mammary tumors (**d**) in the hybrid (FVB/N × C57BL/6) C3(1)/TAg transgenic mice carrying mutant *bax* alleles. (**a**) Kaplan-Meier analysis of survival rates showed significant differences in survival between the $bax^{+/+}$ and $bax^{+/-}$ groups (**$P<0.01$). (**b**) The incidence of the mammary tumors was significantly increased in $bax^{+/-}$ mice during 19–21 weeks of age as compared to $bax^{+/+}$ mice (*$P<0.05$). (**c**) Multiplicity of the mammary tumors was much higher in $bax^{+/-}$ mice than in $bax^{+/+}$ mice. (**d**) Tumor volume tended to be larger in $bax^{+/-}$ mice compared to $bax^{+/+}$ mice. Data presented as mean ± s.d. Ten mice with $bax^{+/+}$ genotype and 16 mice with $bax^{+/-}$ genotype were examined. X-axis begins at 13 weeks of age (**a**–**c**). Figure reprinted with permission of Oxford University Press from the original article by Shibata *et al*., 1999, published in *The EMBO Journal*

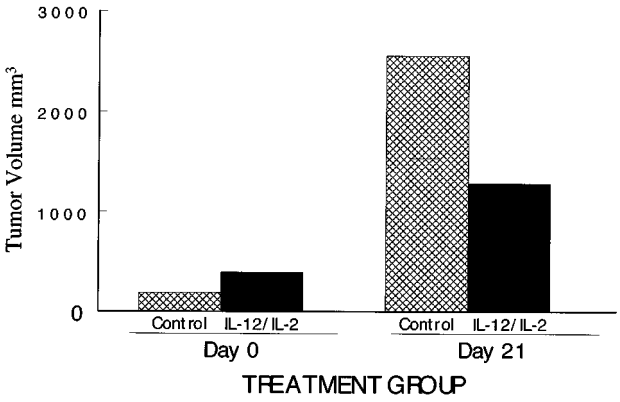

Figure 6 Impact of administration of IL-12/pulse I1-2 on growth of C3(1)/Tag mammary carcinomas

tumor growth, increased tumor volume and decreased survival (Figure 5; Shibata *et al*., 1999). These results confirmed that the level of Bax expression plays an important role in modulating tumor progression in this model.

Reduced expression of Bax does not affect the rates of cellular proliferation during any stage of mammary tumor progression. However, reductions in Bax expression lead to a significant decrease in the rate of apoptosis, but only during the preneoplastic stage of tumor formation (Shibata *et al*., 1999). The expression of Bax in this mammary cancer model is not dependent upon p53 expression, unlike studies performed in a transgenic model in which choroid plexus tumors develop (Yin *et al*., 1997). This suggests that the regulation of *bax* by p53 may be tissue-specific.

Ongoing studies will attempt to determine what mechanisms are responsible for suppressing apoptosis as lesions transition into invasive carcinomas.

Testing therapeutic approaches

Since many biologic differences exist between the mouse and the human, mouse models need to be validated for their usefulness as systems in which to test cancer therapies that may be applicable to human breast cancer. We have examined the therapeutic efficacy of several approaches using the C3(1)/Tag transgenic model.

Chemoprevention agents reduce tumor development The ideal choice for reducing cancer morbidity and mortality is cancer prevention. Most chemoprevention studies to date have been preformed using chemical carcinogenesis models that have certain limitations. The molecular targets and their relevance to human cancer in chemically induced transformation are not well characterized. The mammary tumor phenotypes from such models generally do not have histologic characteristics which resemble human breast cancer (see article by Cardiff *et al*., in this issue), suggesting that the targeted cell compartment or oncogenic pathway may be different than those in human breast cancer.

Since the C3(1)/Tag transgenic mammary model shares several important features with human breast cancer, we have tested whether established chemopreventive agents are able to alter the natural history of tumor development in this model. The results of these studies demonstrate that both di-fluoro-methyl ornithine (DFMO), hi-hydroepiandrosterone (DHEA) (Green *et al*., manuscript in preparation), and 9-*cis* retinoic acid (Wu *et al*., submitted) are able to significantly reduce the incidence, multiplicity and volume of tumor growth in this transgenic model. This transgenic model may be useful for screening other selected chemopreventive agents for their efficacy in inhibiting the development and/or progression of mammary cancer.

Immunotherapy: regression of tumors using IL-2/IL-12 pulse therapy Since the direct targeting of tumor cells using gene therapy approaches has several important technical limitations, we have focused on approaches in which the immune system may be stimulated to destroy cancerous cells in this transgenic model. The advantage of using a transgenic model for these types of studies over xenograft models is that tumors arise spontaneously in the appropriate organ in an animal. The immune system is conditioned by the entire process of tumor development and progression.

Various efforts to overcome immune tolerance to the SV40 Tag expressed in this model by vaccination have been unsuccessful. Tumor cell lines developed from the mammary tumors fail to elicit anti-tumor protection despite the engineered overexpression of granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-2 (IL-2), and the co-stimulatory molecule B-7 with or without the enhancing effect of co-treatment with anti-CTLA-4 (unpublished data). These approaches have, however, been successful when applied to xenograft systems (Hodi and Dranoff, 1998; Hurwitz *et al*., 1998).

In contrast, experimental trials using IL-12 pulse IL-2 combination cytokine therapy for the transgenic mammary tumors have resulted in a significant impairment of tumor progression (Figure 6) and regression of the majority of established tumors (Table 1; Wigginton *et al*., submitted). Interestingly, this effect does not appear to involve immune memory as new tumors develop at different sites within the mammary glands of mice where tumors regressed following termination of the IL-2/IL-12 therapy. This dramatic anti-tumor response may be mediated, in part, by anti-angiogenic effects of IL-12 (Voest *et al*., 1995). Recent unpublished studies have also demonstrated that the anti-angiogenic compound endostatin can reduce mammary tumor incidence and growth in this model (S Ramakrishnan, manuscript in preparation).

Summary

The C3(1) regulatory sequences direct Tag expression to the ductal epithelial and TDLU in virgin animals. This transgenic model for mammary cancer demonstrates several important features that are similar to those observed in human breast cancer. These include the progressive histologic development of carcinomas in 100% of female mice (resembling lesions described as human infiltrating ductal carcinomas) over the course of several months, passing through an

intermediate stage which resembles human DCIS. Genomic and molecular alterations during stages of tumor progression can be studied. The Tag-induced transformation process leads to alterations in the expression of cell cycle genes as are similarly observed in human breast cancer. This model appears useful for testing novel therapeutic strategies.

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